SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE S1. Comparison of the switch II sequences of mouse and human Rab family members. Sequence alignment of the switch II region of mouse or human Rab1–43 (see Ref. 19; GenBank™ accession numbers AB232583–AB232642). Conserved residues are shown against a black background. The positions of two highly conserved amino acids in the switch II region of Rab34 and Rab36 are shown against a red background.

SUPPLEMENTAL FIGURE S2. Endogenous Rab36 protein and RILP protein are localized on mature melanosomes. *A*, Immunoaffinity purification of mature melanosomes from melan-a cells with anti-tyrosinase IgG-conjugated magnetic beads was performed as described in the *Experimental Procedures*. Melanosomal fractions were analyzed by 10% SDS-PAGE followed by immunoblotting with the antibodies indicated. The amount of IgG heavy chain (HC) used for immunoprecipitation is shown in the bottom blot. Input means 1% of the volume of the crude membrane fractions used for immunoaffinity purification (lane 1). Note that Rab36 was co-purified with melanosome markers (lane 3 in the top three panels), but not with any of the other organelle markers. The positions of the molecular mass markers (in kilodaltons) are shown on the left. *B*, immunostaining of RILP in a control melan-a cell and in *RILP* shRNA-transfected cells. Note that some RILP signals were clearly observed on melanosomes (arrowheads in the insets; melanosomes are pseudo-colored in green) in the control cell, whereas the signals were dramatically diminished in the RILP knockdown cells (marked by EGFP fluorescence in the bottom panel). The insets show magnified views of the boxed areas. Scale bars, 10 μm.

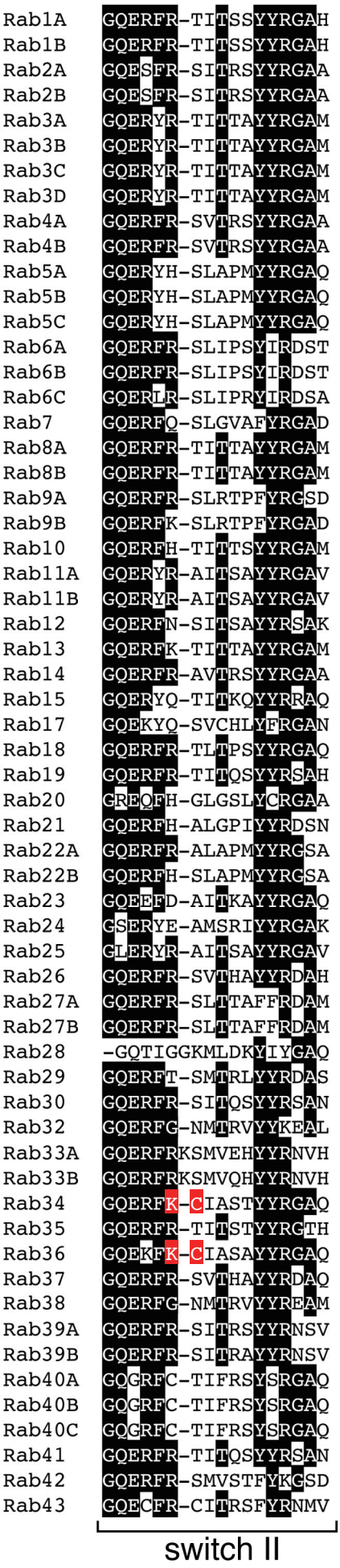
SUPPLEMENTAL FIGURE S3. Distinct requirement of Lys-120 and Cys-121 in the switch II region of Rab36 for binding to Rab36-binding proteins. Yeast cells containing the pGAD (or pAct2) plasmid expressing one of the Rab36-binding proteins indicated and pGBD plasmid expressing Rab36(CA)/WT or Rab36(CA)/(K120A/C121A) were streaked on SC-LW (left panel) and SC-AHLW (selection medium; right panel) and then incubated at 30˚C for one day and two days, respectively.

SUPPLEMENTAL FIGURE S4. Rab36-binding proteins identified by our yeast two-hybrid screening interact with Rab36 in COS-7 cells. Associations between T7-tagged Rab36-binding proteins (Gripap1, Appbp2, Ehbp1L1, GAPCenA, JIP3, and JIP4) and FLAG-tagged Rab36 in the presence of 0.5 mM GTPγS were analyzed by co-immunoprecipitation assays with anti-FLAG tag antibody-conjugated agarose beads (or anti-T7 tag antibody-conjugated agarose beads; for JIP3 and JIP4) as described previously (13, 27). Proteins bound to the beads were analyzed by immunoblotting with the antibodies indicated. Note that except for Ehbp1L1 all of the Rab36-binding proteins tested also bound Rab36 in mammalian cells. Input means 1/80 volume of the reaction mixture used for immunoprecipitation (top panel). The positions of the molecular mass markers (in kilodaltons) are shown on the left.

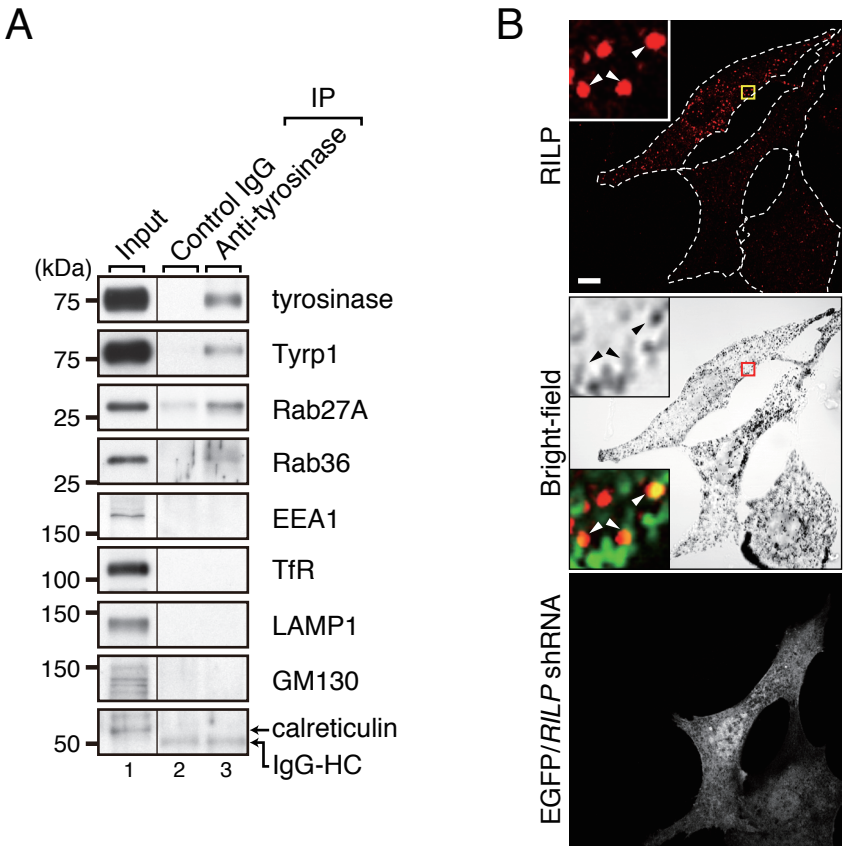
SUPPLEMENTAL FIGURE S5. Low magnification views of bright-field images of

mStr-RILP (WT or mutants)- and EGFP-Rab36-expressing cells. Note that mStr-RILP (WT, E233A, or N235A)- and EGFP-Rab36-expressing cells (outlined with a broken red line) exhibited perinuclear melanosome aggregation (see also Fig. 7A), whereas the surrounding untransfected cells exhibited a normal peripheral melanosome distribution. Scale bars, 10 μm.

SUPPLEMENTAL FIGURE S6. Expression of mStr-RILP mutants and EGFP-Rab36 mutants in melanocytes. *A*, equal protein expression levels of wild-type (WT) and mutant RILP and EGFP-Rab36 in melan-a cells. Melan-a cells were transfected with pEGFP-C1-Rab36 and pmStr-C1-RILP (WT, L231A, E233A, R234A, or N235A). Two days after transfection, the cells were harvested and lysed with a lysis buffer (50 mM HEPES-KOH, pH 7.2, 150 mM NaCl, 1 mM $MgCl_2$, 1% Triton X-100, and appropriate protease inhibitors). The cell lysates obtained (10 μg each) were subjected to 10% SDS-PAGE followed by immunoblotting with anti-RFP antibody (1/1000 dilution, top panel), anti-GFP antibody (1/5000 dilution, middle panel), and anti-actin antibody (1/10,000 dilution, bottom panel). Since the level of expression of each mStr-RILP mutant protein was equal to that of the wild-type protein, the reduced melanosome aggregation activity of mStr-RILP(L231A) and mStr-RILP(R234A) (Fig. 7B) cannot have been attributable to insufficient expression of the mutant protein. *B*, similar protein expression levels of wild-type and a mutant EGFP-Rab36 in melan-a cells. Melan-a cells were transfected with pEGFP-C1-Rab36 or pEGFP-C1-Rab36(K120A/C121A), and two days after transfection cell lysates were prepared and analyzed as described above. Since the levels of expression of EGFP-Rab36 and EGFP-Rab36(K120A/C121A) were similar, the reduced melanosome aggregation activity of EGFP-Rab36(K120A/C121A) (Fig. 7D) cannot have been attributable to insufficient expression of the mutant protein. The positions of the molecular mass markers (in kilodaltons) are shown on the left.



Matsui *et al.*, Suppl-Fig.1, Top ⇑



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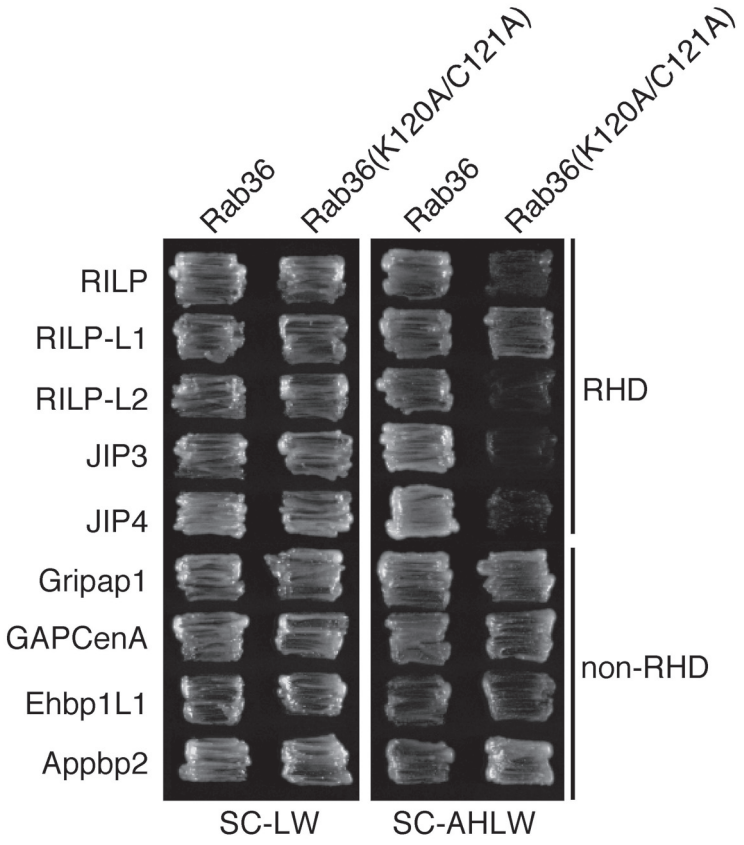

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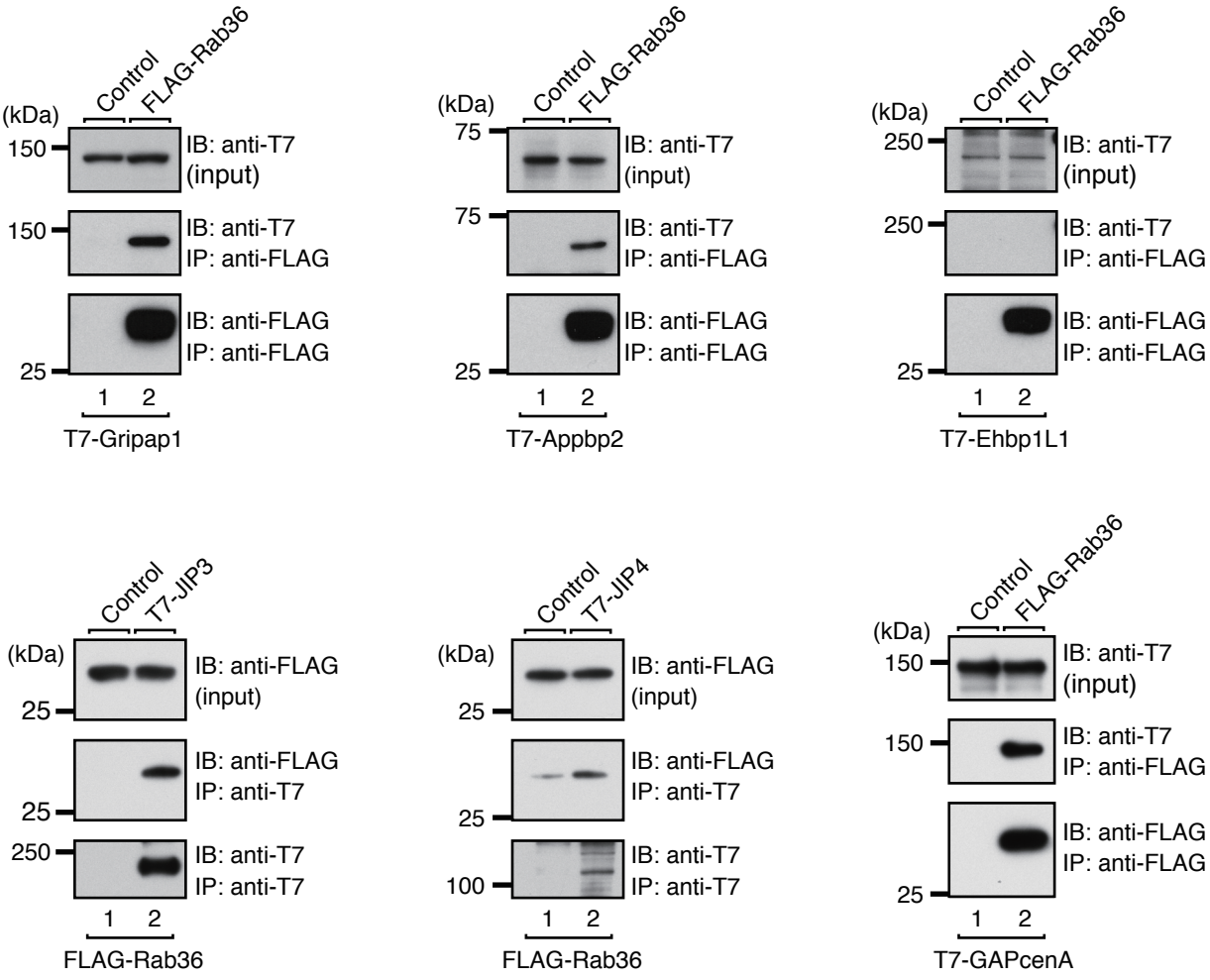

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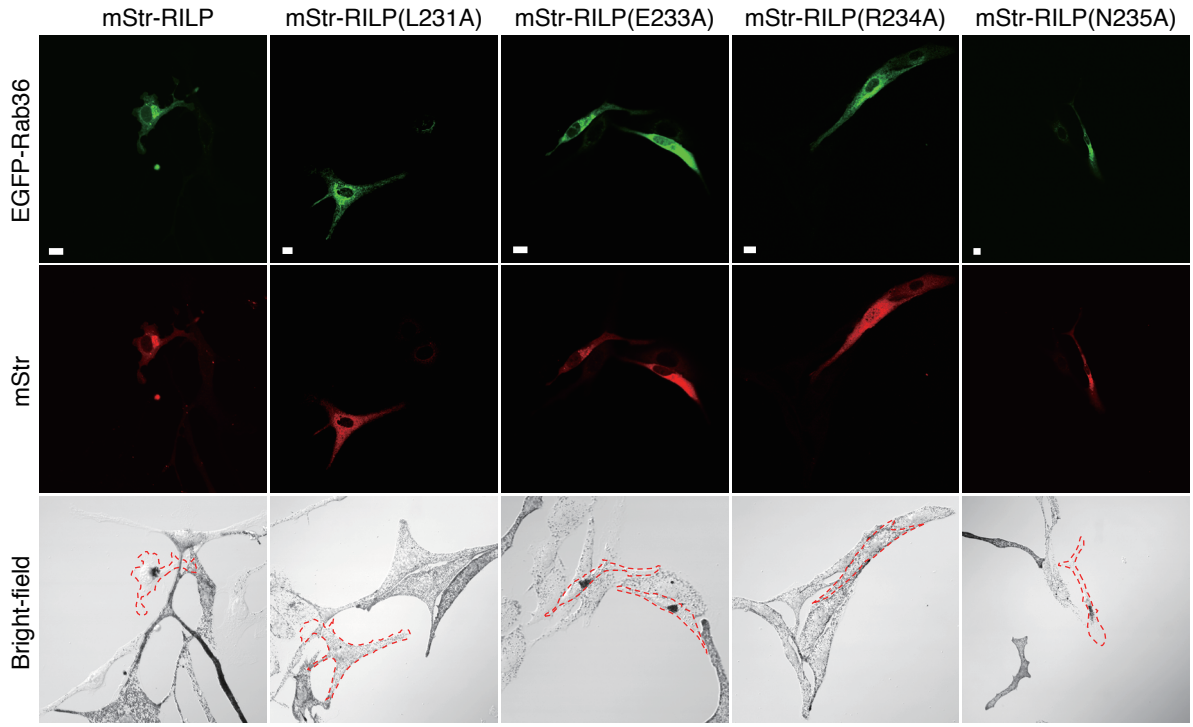

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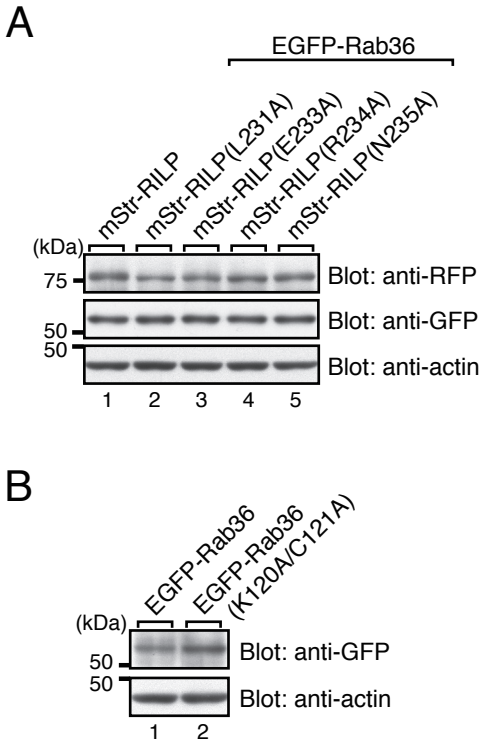

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